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Impact of hypofunctional occlusion on upper and lower molars after cessation of root development in an adult mouse

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**Impact of hypofunctional occlusion on upper and lower molars after
cessation of root development in an adult mouse.**

Abstract:

Background: Hypofunctional occlusion is known to lead to changes in the length of roots over time, the mechanisms that drive such changes, however, are poorly understood, with most studies concentrating on juvenile rats prior to the arrest of root development. In this manuscript we investigated the response of the upper and lower first molar roots to lack of occlusion concentrating on time-points after the development of the roots has ceased using the mouse as a model. Mouse molar roots finish development at weaning, much earlier than rat molars, and display a similar pattern of roots in the lower and upper jaw to humans.

Methods: Hypofunctional occlusion was achieved in adult mice at 5 and 9 weeks of age by flattening the upper 1st molars cusps. Mice were then sacrificed after 6 and 2 weeks respectively along with control littermates. microCT was used to measure root length, alveolar bone height, and the amount of tooth eruption, followed by sectioning to understand the mechanisms behind the changes at the histological level.

Results: In the lower first molar the response to hypofunctional occlusion was characterized by elongation of both the mesial root and its surrounding alveolar bone, while the distal root was unaffected. In contrast, the response of the upper first molar was characterized by over-eruption of the mesial side of the tooth without any significant change in the alveolar bone or root length. From histologic sections it was clear that increased deposition of cellular cementum played an important role in the changes that occurred in the lower mesial root.

Conclusions: In a mouse model, upper and lower molars responded differently to hypofunctional occlusion, with adult mice showing a different response to that previously reported for juvenile rats, highlighting the importance of considering age and tooth position in cases of hypofunction.

Introduction:

A tooth is composed of two parts: the crown and the root, with root formation starting after crown morphogenesis has completed. The process of root formation is complex involving interaction between the ectoderm and neural crest derived mesenchyme (1). Root development starts by elongation of the inner and outer enamel epithelium through the underlying mesenchyme forming Hertwig's epithelial root sheath (HERS). As the HERS elongates, the cells of the dental papilla adjacent to the HERS differentiate into pre-odontoblast cells (2) before forming odontoblasts, which later secrete the dentin layer (2, 3). The dentin in the root is covered by a layer of cementum that can be divided into two types, acellular and cellular. Acellular cementum, which is composed of cementum matrix only, forms at the beginning of root development and covers the cervical and middle 3rd of the root. The cellular cementum is deposited through out life mainly at the apical 3rd of the root; it contains cementoblast cells that are trapped in the cementum matrix giving its distinctive appearance.

Although the embryonic origin of the cementum layer has been studied, it remains controversial. Lineage labeling studies have shown that cementoblasts are derived from neural crest cells (4, 5), however other studies have suggested the HERS can undergo epithelial-mesenchymal transition and contribute to the formation of cementoblasts (6-9). It has also been suggested that acellular and cellular cementum derived from different origins; the acellular derived from HERS and the cellular from neural crest cells (6). A contribution from both the neural crest and from the epithelium appears likely. During adult life, cementum deposition can occur in response to local factors such as trauma, infection, change in occlusal forces, or in response to systemic factors such as aging or in response to diseases such as Paget's disease, acromegaly, and Pituitary gigantism. The consequence of such hypercementosis is ankylosis leading to difficulty in extraction of teeth and poor orthodontic movement (7-9).

When a tooth crown comes in contact with the opposing tooth crown occlusion is established. In humans, an ideal occlusion is rare; therefore orthodontic and surgical treatment may be required to provide a better occlusion especially for those who have severe aesthetic and oral function problems. Several studies have investigated the response of different tooth tissue components when different forces are applied to them but the results obtained were variable. Some showed that there was loss of connective tissue and gingival recession during tooth extrusion (10), while others stated that no changes in the periodontium was noticed in teeth

1 which were impacted and extruded orthodontically (11). Pathological changes including
2 degeneration and disruption of odontoblast layer and dilatation and congestion of blood
3 vessels had been reported to follow tooth extrusion (12, 13) but other reports have shown that
4 neither the pulp nor the dentin undergo any pathological changes during extrusion (14).
5 The use of animal models is now has been widely accepted and has contributed to the
6 understanding of not just diseases but also the consequences of specific treatments. Given this,
7 orthodontic treatment can be investigated in animal models to find out the changes that might
8 occur in human teeth.
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16 Given that hypofunctional occlusion is widely applied during orthodontic treatment, research
17 has been carried out to follow the consequence of hypofunctional occlusion. A variety of
18 methods have been employed to achieve hypofunctional occlusion involving insertion of
19 blocking devices or removal of opposing teeth. Bite blocks applied on rats was shown to lead to
20 a decrease in alveolar bone volume and acceleration of tooth movement as a consequence of
21 hypofunction (15, 16). The use of bite-raising appliances in 5 weeks old rats resulted in an
22 elongation and over eruption of the mesial root of the upper 1st molars after a 6 weeks period
23 of hypofunction (17). Extraction of the 1st and 2nd lower molars on one side of the jaw before
24 eruption led to an elongation of the mesial root of the upper 1st molar driven by increased
25 proliferation of HERS (18). This study also reported hypogenesis of the cementum during root
26 elongation. Rather than whole tooth extraction, hypofunctional occlusion has also been induced
27 by removal of the crown of the upper molars on one side of 5 week old rats to assess the effect
28 on the opposite lower molars (19). This study reported elongation and over eruption of the
29 lower molar roots and a decrease in the height of the alveolar bone. Interestingly age appeared
30 to be a key factor in such experiments with lower molars becoming more suprapositioned in
31 young rats aged (4 weeks) than in adults (26 weeks) when the crown of the upper molars was
32 reduced (20), in these experiments, the alveolar bone height was higher in young rats compare
33 to the controls while no difference was found in adults.
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48 With the exception of the last study, hypofunctional occlusion has mainly been studied in young
49 rats prior to the completion of root development and the cessation of the HERS, which occurs
50 around 7 weeks after birth (postnatal day P50)) (21). Therefore many of the experiments were
51 conducted while normal root development was still occurring. We decided to investigate the
52 consequences of hypofunctional occlusion in mice, where root development stops much earlier,
53 around P20 (22). This allowed us to specifically look at the influence of occlusion after the
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1 cessation of root development. Importantly, the previous adult rat paper (20) lacked
2 histological evidence to understand the mechanism of root elongation, therefore, we sectioned
3 our adult molar roots to understand how any changes in the root and bone are generated.
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7 Another advantage of mice is that they have the same number of roots as humans, 3 roots in the
8 upper first and second molars (M^1 , M^2), and 2 roots in the lower first and second molars (M_1 ,
9 M_2) making them a better and simpler model when compared to rats which have more roots;
10 upper 1st molar (M^1) has 5 roots, upper 2nd molar (M^2) has 4 roots, upper 3rd molar (M^3) has 3
11 roots and lower 1st molar (M_1) has 3 roots (19, 23). These differences in root number may
12 influence the response of the tooth generally and the roots specifically to hypofunctional
13 occlusion.
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19 Previous studies have investigated either upper or lower teeth so it is not known whether there
20 is any difference in the response of the two (upper and lower teeth) during hypofunctional
21 occlusion. Importantly in this paper we will investigate the response of the upper and lower
22 teeth to a loss of occlusion force using both μ CT and histology in order to distinguish the
23 mechanism that derive any change in tooth shape.
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1 **Materials and methods:**

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4 **Alteration to Occlusion:**

5 Litters of 5 weeks old mice were divided into 2 groups; the 1st group was a control (N= 6
6 animals) and the 2nd group underwent flattening of the cusps of upper 1st molar on both sides
7 of the jaw using a FG1/4 round bur and dental hand piece without exposure of the pulp (N = 4
8 animals). Treatment was stopped once all cusps were removed. The procedure was carried out
9 while the mice were under general anesthesia using Hypnorm: Water: Hypnovel (midazolam
10 10mg/2ml) at a ratio of 1:2:1. For every 10mg of body weight 100µL of the prepared solution
11 was injected intraperitoneally. After every 2-3 seconds of drilling the tooth was cooled down
12 with a small cotton roll dipped in a sterile 1x PBS. The control littermates did not undergo
13 surgery. Flattening of cusps was restricted to the upper 1st molars on both sides of the jaw as
14 the angle of the head made accurate lower molar operations impossible. Both groups were fed
15 on a mash diet (RG3-E-FG Expanded ground Special Diets Service) throughout their life i.e.
16 before and after the grinding procedure. The samples were sacrificed after 6 wks from the time
17 their teeth were flattened by cervical dislocation. The same experiment was repeated on mice
18 aged 9 weeks (N = 4 animals), who were sacrificed 2 weeks after the procedure. Mice at 9
19 weeks and 5 weeks can be considered adult as their roots have stopped growing (22) and they
20 are sexually mature.

21 To take into account any changes in body weight as a consequence of tooth grinding, a second
22 set of mice were weighed before treatment and again at 2 and 6 weeks post operation. All the
23 mice gained weight within the normal range of CD1s (according to growth chart provided by
24 Harlan), with no effect of grinding on overall weight, compared to non-ground littermates
25 (student T-test >P:0.05). These results agree with previously published work where
26 hypofunctional occlusion was not found to influence body size (15,16), and implies that the
27 mice did not have a reduced dietary input as a consequence of pain induced by the grinding.

28 Mice were housed in the biological service unit (BSU) of King's College London. All animal
29 procedures were covered under Home office licenses and all animals used in the experiments
30 were culled using schedule 1 methods

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53 **MicroCT (Microcomputed Tomography):**

54 Mice from all the groups were scanned using a GE Locus SP microCT scanner. The specimens
55 were immobilized using cotton gauze and scanned to produce 14µm voxel size volumes, using
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an X-ray tube voltage of 80kVp and a tube current of 80 μ A. An aluminum filter (0.05mm) was used to adjust the energy distribution of the X-ray source. To ensure scan consistency, a calibration phantom of known geometry (a dense cylinder) was positioned within the field of acquisition for each scan. We then carried out test reconstructions on this object to determine the optimum conditions for reconstruction. This allowed the centre of rotation to be determined precisely, ensuring consistency in image quality, minimising blurring and therefore maximising detection of object movement.

The specimens were characterized further by making three-dimensional isosurfaces, generated and measured using Microview software (GE).

Measurements of MicroCT samples:

1. Measurement of alveolar bone height:

In the lower jaw, the alveolar bone height was measured using two methods. Firstly we measured from the alveolar crest to the base of the mandible at the buccal side of a coronal section, at an area mesial and parallel to the mesial root of the lower 1st molar. As an alternative method, based on previous published results (20), we measured from the centre of the mandibular canal to the alveolar crest from the lingual and buccal sides of the jaw in coronal section. These measurements were performed in both the controls and the samples with hypofunctional occlusion (ground samples) to allow a comparison (Fig. 1A).

In the upper jaw, the alveolar height was measured from the alveolar crest (next to the mesial root of the 1st molar) to the base of the zygomatic arch where the mesial root is anchored (Fig. 1B).

2. Measurement of root length and quantification of tooth eruption:

Root length in both the upper and the lower 1st molars was measured from the Cemento-enamel junction to the root apex in sagittal section (Fig. 1C). As the upper molar cusps were flattened it was not possible to measure from the cusps to the roots for the upper molars. This measurement was therefore not used for the lower molars, as we wished to have a consistent methods for use on both lower and upper teeth. For the upper 1st molar, all three roots were measured (mesial, palatal and distal); while for the lower 1st molar both roots were measured (mesial and distal). The amount of eruption of the mesial root of both upper and lower 1st

1 molar was calculated by measuring the area from the cemento-enamel junction to the alveolar
2 crest when viewed by coronal section at the buccal side (Fig. 1D).
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6 **3. Measurements of mesial root in cross section:**

7 The width and length of the cross-sections of the lower 1st molars mesial root was measured
8 using microCT using two different methods. Firstly we used a subjective measure where we
9 measured at the junction of the cervical and middle 3rd of the root, and at the junction of the
10 middle and apical 3rd (Fig. 3G). This measurement means relative differences in root length
11 were not taken into consideration, and is useful when dealing with teeth of variable size. We
12 then used a more objective method choosing levels corresponding to 0.65mm and 1.25mm
13 from the bifurcation point down the mesial root. For the cross sectional measurements,
14 sections at 90 degrees through the root were taken (schematically shown in Fig. 3G). This was
15 confirmed by viewing the tooth in 3D to show the angle of the plane (images shown in Fig. 3C,D
16 rotated to view in Fig. 3A,B). Having confirmed the angle of the plane, we drew a standard
17 angled cross on the exposed root (see Fig. 3C,D), with the centre at the middle of the root, and
18 from this measured the width and length of the root. The ratio between width and length at
19 both levels were compared in unoperated and ground individuals.
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31 **Histology sections:**

32 The samples were fixed in 4% PFA for 3 days then decalcified in a solution made of 67.5%
33 EDTA, 25% PFA, and 7.5% PBS for 2 months. The decalcified solution was changed twice a
34 week. After decalcification the samples were dehydrated through a Methanol series. Then
35 washed in isopropanol, cleared in 1,2,3,4 Tetrahydronaphthalene and embedded in paraffin
36 wax. The samples were sectioned with a microtome at 10µm thickness. The slides were then
37 stained using picro-sirius red, haematoxylin and alcian blue.
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45 **Statistical analysis:**

46 All data was checked to confirm it followed a normal distribution before an unpaired student t-
47 test was used to compare the ground and control samples. For most of the data we used a
48 Shapiro-Wilk test, except for data sets with a number of identical data points in which case we
49 used the D'Agostino-Pearson (omnibus K2 test). A normal distribution was observed in all
50 cases. Prism 6.0 software was used to generate the normality tests and student t-tests. P value <
51 0.05 was considered to be significant.
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Results:

Elongation of the mesial root of the lower 1st molar tooth after hypofunction:

To study the consequence of adult hypofunctional occlusion 5 weeks old mice were chosen for the study, two weeks after the completion of root development. The upper 1st molar's cusps were flattened preventing contact with the lower 1st molar in half the littermates and the mice were sacrificed after 6 weeks, aged 11 wks. Analysis of microCT scans revealed that only the mesial root of the lower 1st molar was significantly elongated (Fig. 2A-C). The mean of the root length in controls was 1.64 mm (N=12 molars) and in the ground samples (samples with hypofunctional occlusion) was 2.07 mm (N = 8 molars) with a P value of <0.0001. No other roots of the 1st molar were significantly altered (Fig 2C). In order to see whether we could identify a significant root elongation at an earlier time point the experiment was repeated but this time using samples aged 9 weeks. In these samples the upper 1st molar was flattened and then the animals were sacrificed after 2 weeks (N=8 molars) and compared to 11 weeks old controls (N= 12 molars). Again a significant difference was found between the two groups for the mesial root (Fig. 2C). The mean of the root length in ground samples after 2 wks of hypofunctional occlusion was 1.84 mm compared to 1.64 mm in the controls with a P value of 0.0009. When we compared both ground samples; those left for 2 wks compared to those left for 6 wks there was a significant difference between the two groups (P value 0.0022). The longer the period that a tooth undergoes hypofunctional occlusion, therefore, the longer the extension of the mesial root observed. These results show that changes in length of roots can occur very rapidly even after a short period without occlusion. In contrast to the lower 1st molar the upper ground 1st molars did not display any significant difference when length and width of all the roots were assessed in comparison to controls (data not shown), indicating different mechanism at play for upper and lower teeth.

Elongation of the root by deposition of cementum:

In order to investigate the mechanism involved in the elongation of the mesial root of the lower 1st molar, the controls and the ground samples were decalcified and processed for histology. The sections revealed that the elongated lower molar roots of the ground samples had more cellular cementum deposition at the lower end of the root (Fig. 2E,G) compared to the controls (Fig. 2D,F). No extension of the dentin part of the root was observed as has been shown when hypofunction was induced before cessation of root development (18). This suggests that

1 deposition of cellular cementum is responsible for the elongation of the root of the lower 1st
2 molar when the root is no longer able to extend by HERS growth, raising the tooth in an
3 attempt to re-establish the occlusion with the opposite 1st molar.
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7 **Widening of the mesial root of the lower 1st molar tooth after hypofunction:**

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9 In addition to a change in root length, the microCT analysis also indicated a change in the shape
10 of the lower mesial root, particularly at the lower part of the root, which appeared wider when
11 viewed sagittally in 3D after 6 weeks of hypofunction (Fig. 3A,B). Cross sections through the
12 apical part of the root, at 90 degrees to the axes of the tooth, showed that the ground roots
13 displayed an elliptical morphology compared to the more rounded morphology observed in the
14 control samples (Fig. 3C,D). To quantify this shape change the ratio of the width to length was
15 compared in cross section of the mesial root in both ground and control teeth. Shape change
16 was compared in two regions of the root, first at the junction of the cervical and middle 3rd of
17 the root, and second at the junction of the middle and apical 3rd of the root (Fig. 3G). A
18 significant difference in the proportion of width to length in the middle to apical 3rd of the root
19 was observed (P value 0.0345) (Fig. 3I). Further towards the crown no difference was observed
20 in the shape of the root, which was rounded in both cases (near to a value of 1) (P value
21 0.1144) (Fig. 3J). Similar results were generated when we compared the cross section at
22 0.65mm and 1.25mm along the mesial root from the bifurcation (Fig. 3K,L).
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35 In order to understand this change in shape, the roots were assessed by histology in transverse
36 section. In the ground roots, cementum was deposited heavily asymmetrically around the root
37 compared to controls, where the cellular cementum was deposited more evenly (Fig. 3E,F). In
38 particular the ground specimens showed large deposition of cementum on the inner surface of
39 the mesial root (Fig. 3H). The shape change was therefore generated by a change in the
40 deposition of cementum rather than any change in the layout of dentine.
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46 **Over eruption of the mesial root of the upper 1st molar and increase in alveolar bone**
47 **height around the mesial root of the lower 1st molar:**

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49 As previous studies have reported over eruption of the upper molars after hypofunctional
50 occlusion (17) we therefore used microCT to assess the degree of over eruption in our samples
51 after 6 weeks of hypofunction. When the distance from the Cemento-Enamel Junction (CEJ) of
52 the mesial root to the alveolar crest was measured, a significant difference was found between
53 the hypofunctional group and the controls for the upper 1st molar (Fig. 4A, B). The same
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1 measurement was carried out on the mesial root of the lower 1st molar, an unexpected decrease
2 in eruption was found in the ground samples compare to the controls (Fig. 4A, B). This finding
3 agrees with previous research that the mesial root of the upper 1st molar over erupts during
4 hypofunctional occlusion; however, our data suggests that the lower 1st molar responds in a
5 very different way. To understand the decrease in eruption observed in the lower 1st molar we
6 next measured the height of the alveolar bone around the mesial root of the lower 1st molar in
7 coronal section using two different points of measurement to ensure our results were accurate
8 (Fig. 1A). We found that the alveolar bone height was significantly increased in the ground
9 samples (hypofunctional samples) compare to the controls, using either point of measurement
10 (Fig. 5C). However when the same measurement was carried out around the mesial root of the
11 upper 1st molar compared to control samples no change was found between the two groups
12 (Fig. 5C). This result demonstrates that during hypofunctional occlusion the alveolar bone
13 height increases in the lower jaw, while in the upper jaw the alveolar bone height remains
14 constant, resulting in supraposition.
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1 **Discussion:**

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4 In our samples we achieved hypofunctional occlusion by surgically flattening the cusps of the
5 upper 1st molar. Unfortunately the angle of a mouse head prevented the same procedure being
6 carried out for the lower molars, and therefore we were unable to investigate the effect of
7 grinding on root length and tooth movement. As our grinding experiments did not damage the
8 tooth pulp the surgery should not have had a direct effect on the health of the tooth and its
9 subsequent response to hypofunctional occlusion. Given this, the differences we observed
10 between upper and lower teeth may represent real differences in response, rather than a
11 consequence of whether the tooth was ground down or not. Our results show that
12 hypofunctional occlusion was characterised by an elongation of the mesial root of the lower 1st
13 molars, and a corresponding increase in the height of the surrounding alveolar bone. This
14 increase in root length was observed even after 2 weeks post grinding. Such an increase in the
15 height of the alveolar bone has previously been reported in the lower jaw in young rats with
16 hypofunctional occlusion, although older rats appeared not to respond in this way (20).

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28 In contrast in the upper jaw we observed an over eruption of the 1st molars without an increase
29 in the alveolar bone or increase in length of the root. The effect on the upper jaw might be
30 influenced by gravity, which could bring the upper tooth downward without the need for
31 significant root elongation.

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36 In our experiment we performed hypofunctional occlusion on both sides of the upper jaw,
37 rather than on one side only. This enabled us to potentially have more similarity with human
38 cases where both sides often undergo hypofunctional occlusion during orthodontic treatment,
39 and avoided possible compensation by developing a preferential side. In patients a preferential
40 side can develop due to malocclusion or pain (24, 25). In hypofunctional tests on rodents both
41 approaches have been performed with some researchers using one side as the experimental
42 side and the other as the control (18), while others applied hypofunctional occlusion to both
43 sides and used untreated littermates controls (15, 17). Both approaches therefore appear to be
44 able to generate interesting results.

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53 In many previous papers no histological analysis was carried out to reveal the mechanism
54 responsible for the elongation of the molar roots. Here we show that the mesial root of the
55 lower tooth elongated by deposition of cellular cementum at the apex. This deposition of
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1 cementum appeared to push the tooth upwards in order to make a contact with the opposite
2 tooth and regain occlusion. . This result is consistent with research that has shown
3 hypercementosis in adult monkeys after hypofunctional occlusion (31).
4 Cellular cementum is a hard tissue which is known to be deposited in response to functional
5 demands (26, 27). Cementum, unlike bone, does not remodel, therefore extensive deposition
6 can cause ankylosis.
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12 We also observed a change in the shape of the lower 3rd of the elongated mesial root in the
13 samples with hypofunctional occlusion. Cross section of the roots showed a change from a
14 circular shape in the controls to an elliptically shape in the samples with hypofunctional
15 occlusion. Such changes in the shape indicate that occlusion does not just determine root length
16 but also shape. Histology sections of the controls and hypofunctional samples revealed more
17 cementum deposition on the inner side of the roots after hypofunction at the apical level while
18 in the controls the cementum was laid down at a similar thickness all around the root. The
19 dentin layer appeared unaffected, as would be expected as the experiments were performed
20 after the root was fully formed. The difference in the pattern of cellular cementum deposition is
21 therefore behind the change in the root shape, and may influence the stability of the tooth.
22 We could not find any change in the dimension of the root in cross section at the upper part of
23 the root i.e. at the cervical-middle 3rd junction or at 0.65mm distance from bifurcation. This
24 part of the root is covered by acellular cementum compared to the cellular cementum found
25 apically, suggesting that the shape changes are driven only by changes in cellular cementum. In
26 contrast to the lower molars, the mesial root of the upper molars were shown to over erupt
27 without any change in length. This is in contrast to previous experiments on young rats that
28 showed an increase in root length in the upper jaw (21).
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41 Measuring the ratio of deposition of hard tissue using the cervical-middle 3rd junction and
42 middle-apical 3rd junction or using specific lengths from the point of bifurcation gave the same
43 result in our study on mice. This is perhaps expected as the animals used were from the same
44 litters, had the same background (CD1), were fed on the same type of food and lived in the
45 same environment. Such consistency might not be expected in the human population where
46 root length has been shown to differ according to ethnic group (28-30). Using both
47 measurements therefore has an advantage.
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55 Our experiments were carried out after the completion of root development in mice. Similar
56 hypofunction experiments have previously been carried out in rats but importantly most of
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1 these have been performed during root development. In young rats hypofunction occlusion has
2 been shown to lead to formation of more root tissue (dentin) due to elongation of Hertwig's
3 Epithelial Root Sheath (HERS) (18). We have shown that in contrast to the rat, elongation of an
4 adult mouse tooth deprived of occlusion is due to deposition of cellular cementum at the base
5 of the tooth root. In the adult mouse the HERS would have degenerated, preventing further root
6 extension using this method. It is likely that during hypofunctional occlusion in both mice and
7 rats the same mechanisms are employed, the difference being solely due to the difference in the
8 developmental stage of the roots at the start of the experiments. Hypofunction tests on rats at
9 older ages therefore would be predicted to give similar results to our findings in mice. Our
10 results indicate a difference in the response of the upper and lower molars to lack of occlusion.
11 Previous hypofunction experiments in rats have concentrated on either the upper or the lower
12 first molar, rather than comparing the two in the same experiment and therefore direct
13 comparisons between the response of upper and lower molars has not been made.

22 Over eruption is classed as a projection of the tooth beyond the line of occlusion; however the
23 presence of the diastema region in rodents (an anatomical space between the molars and the
24 incisors) makes it difficult to predict the occlusal plane in the area next to the mesial part of the
25 first molar. Previous papers have calculated the amount of tooth eruption by measuring the
26 distance from the alveolar crest to the mesial cusp tip (19). Here we considered the distance
27 from the alveolar bone to the cemento-enamel junction as the cusp tips were worn off during
28 grinding and therefore could not be used as reliable reference points.

36 Our study has utilised mice for the hypofunction tests as mice have a similar root pattern to
37 humans but also this will enable us to investigate the molecular aspects of hypofunction in
38 transgenic mice in the future. This will allow us to understand the signalling pathways and
39 transcription factors important in tooth movement and root growth and lineage trace the
40 cementoblasts during hypofunction. Only the mesial root of both upper and lower 1st molar
41 responded to lack of occlusion in our experiments. The mesial roots were also more
42 dramatically changed in previous experiments in rats (17, 18). This may be because the mesial
43 root in rodents is next to (the diastema), which may make it easier to adapt in response to
44 applied forces over a relatively short period of time.

53 From our results we concluded that hypofunctional occlusion in adults causes change in the
54 shape and length of the lower molar mesial root by deposition of cellular cementum,
55 accompanied by an increase in the alveolar height, while in the upper jaw the teeth over erupt

without significant elongation or change in the alveolar bone. The teeth therefore use different mechanisms to reach occlusion, something that should be considered when performing orthodontic treatment.

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Figure Legends:

Figure 1: Measurements carried out from 2D μ CT sections.

(A,C) Arrows indicate measurements taken of lower 1st molar and (B,D) measurements on upper 1st molar. (A) Measuring alveolar bone height (two measurements). (B) Alveolar bone length around upper mesial root. (C) Lower root length (D) Amount of eruption of the mesial root of the upper molars.

Figure 2: Mesial root elongation via cementum deposition

(A,D,F) Control molars 11 week old mouse. (B,E,G) Molars after hypofunctional occlusion for 6 weeks in 11 week old mouse.

(A,B) MicroCT 3D reconstructions highlighting the mesial root of the first lower molar in lateral view. Lines indicate apical end of the root. (C) Graph showing difference in root length between control mice and those having undergone hypofunction for 2 and 6 weeks for the mesial root, and 6 weeks only for the distal root. (D-G) Histology sections of the mesial root of the lower 1st molar in control and ground samples. (F,G higher power views of D,E). The white arrows indicate the extent of cellular cementum on the inner side of the mesial root and the blue arrows indicate the extent of cellular cementum on the outer side of the mesial root.

D = dentin, C = cementum, RC = root canal. Yellow dots outline limit of dentin. Scale bars in D-G = 100 microns.

Significance levels in C = ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$

Figure 3: Mesial root shape change at the apical end

(A,B) MicroCT 3D reconstruction of the mesial root of the 1st lower molar in buccal view, showing change in shape at the apical end after hypofunction for 6 weeks. (C,D) 3D reconstruction of the cross section of the root at the level of the middle 3rd and apical 3rd junction (see G) highlighting the change in root dimension at this level. (E,F) Histology section of the root at the level of the middle 3rd and apical 3rd junction of the root. The yellow dots outline the border between the dentin and the cementum. The cementum layer is much more evenly deposited in the controls.

1 (G) Schematic of a 1st molar, the lines indicate the level of the root where measurements of the
2 dimension of a cross section of a root were carried out at first middle 3rd and apical 3rd junction
3 and second is the cervical and middle 3rd junction.
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5 (H) Histology section showing the whole root in a ground sample, highlighting the large
6 amount of cementum deposited on the inner surface of the mesial root compared to the other
7 surfaces of the root. Boarder of dentin and cementum outlined in white. L= = labial, B = buccal,
8 D = distal, M = Mesial.
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10 (I) Graph showing a significant difference in root dimension between the controls and the
11 ground samples (hypofunctional) at the level of the middle and apical 3rd junction. (J) Graph
12 highlights no difference in the root dimension at the level of the cervical and middle 3rd
13 junction between the ground and control samples. (K) Graph showing a significant difference in
14 root dimension between the controls and the ground samples (hypofunctional) 1.25mm from
15 the level of bifurcation. (L) Graph highlights no difference in the root dimension between the
16 ground and control samples 0.65mm from the level of bifurcation.
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18 * = P<0.5
19 D = Dentin, C = Cementum, PDL = Periodontal ligament. Scale bars in E,F,H = 100 microns.
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31 Figure 4: Over eruption of the upper 1st molar after hypofunction
32 (A) 3D reconstruction of upper and lower molars of a control (1st column) and a hypofunctional
33 sample (2nd column). The red line indicates amount of eruption of the mesial root from both
34 the buccal view and palatal view. (B) Graphs showing the impact of hypofunction on eruption of
35 the mesial root of the 1st molar. The lower molar was less erupted while the upper molar was
36 suprapositioned. (C) Graphs showing the impact of hypofunction on the height of the alveolar
37 bone around the mesial root of the 1st molar. The bone around the lower molar was increased
38 while there was no effect on the bone around the upper molar.
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45 * = P<0.5, ** = P<0.01, *** = P < 0.001
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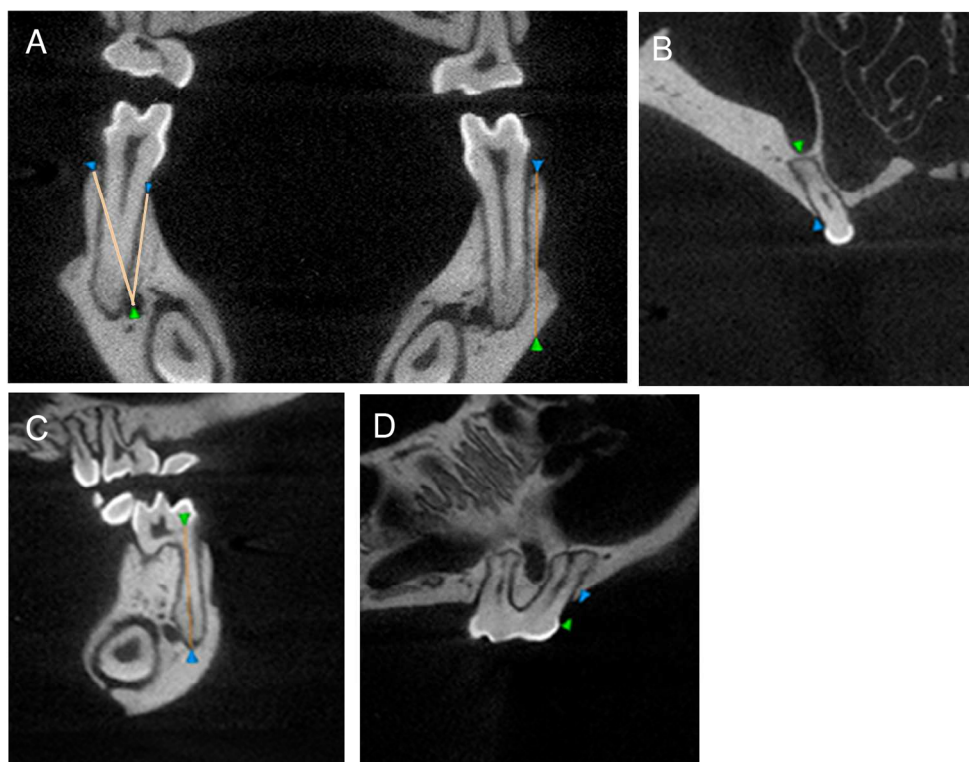


Figure 1

148x115mm (300 x 300 DPI)

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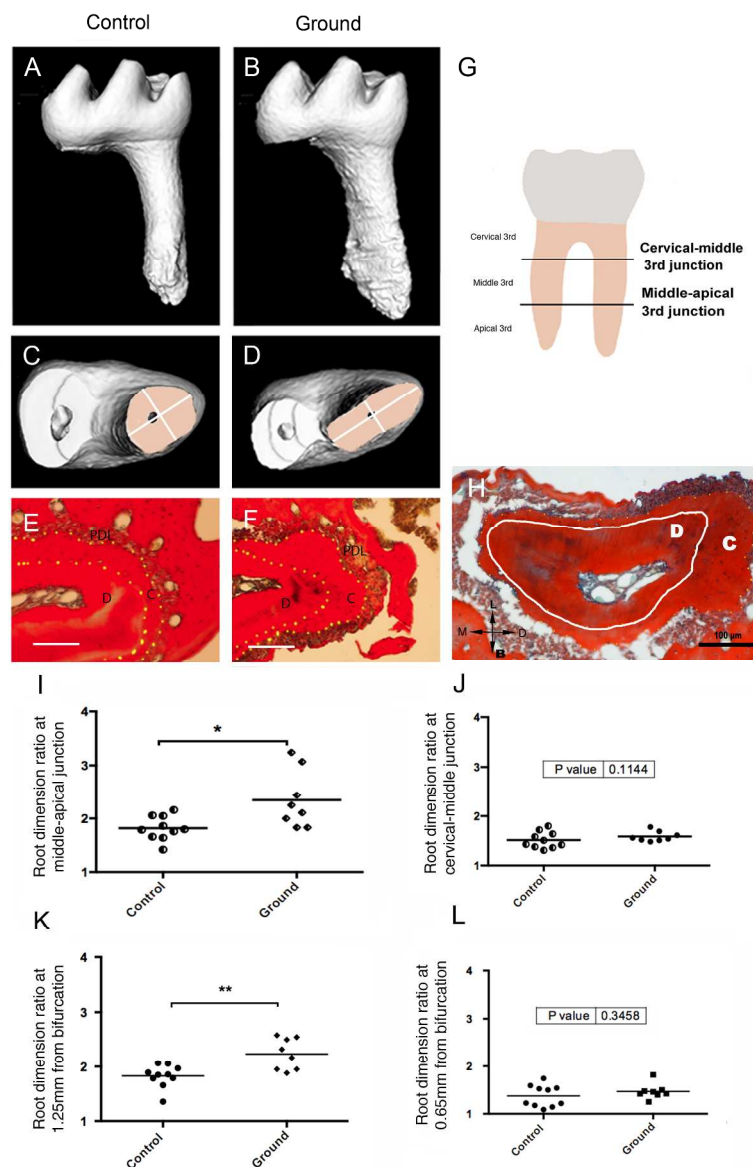


Figure 3

194x297mm (300 x 300 DPI)

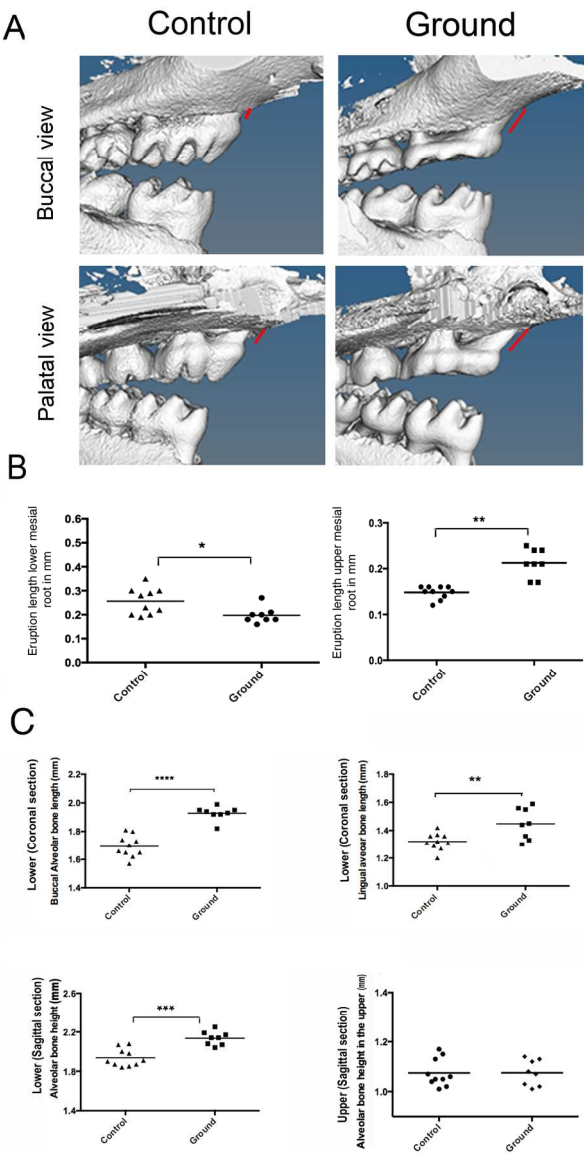


Figure 4

109x213mm (300 x 300 DPI)